# Does Anti *Saccharomyces cervisiae* Antibody (ASCA) distinguish Crohn's Disease from Intestinal Tuberculosis – A Pilot Study

**A dissertation submitted in partial fulfilment of DM (Gastroenterology) course requirements of the Tamil Nadu Dr. MGR Medical University, Chennai**

# Certificate

This is to certify that this dissertation entitled '**Does Anti *Saccharomyces cervisiae* Antibody (ASCA) distinguish Crohn's Disease from Intestinal Tuberculosis – A Pilot Study**' is a bonafide work done by Dr. Amit Kumar Dutta in partial fulfilment of the rules and regulations for DM (Gastroenterology) examination of the Tamil Nadu Dr. MGR Medical University, to be held in August 2008.

Dr. Ashok Chacko,
Professor of Gastroenterology
Clinical Gastroenterology and Hepatology
CMC, Vellore
(Guide)

Dr. Gagandeep Kang,
Pofessor of Microbiology,
Wellcome research unit
CMC, Vellore
(Co-guide)

Dr. B.S. Ramakrishna,
Professor of Gastroenterology
Head, Clinical Gastroenterology and Hepatology
CMC, Vellore

# Acknowledgement

I sincerely thank Dr. Ashok Chacko for his guidance, encouragement and constant support in undertaking and completing this research project. I am indebted to Dr. Gagandeep for her valuable opinions and providing Lab facilities needed to carry out this work. I also thank Mrs. Sheela and other laboratory staff for their help.

# Index

# Introduction

Crohn's disease (CD) is now diagnosed with increasing frequency at many centres in India. Intestinal tuberculosis (ITB) frequently encountered in our country is difficult to distinguish from CD as both diseases have similar radiological, endoscopic and histologic features. Differentiation of CD from ITB is important both for planning treatment and assessing prognosis.

Studies from the west have shown that there is a high prevalence of ASCA in Crohn's disease. They have used this to differentiate Crohn's disease from ulcerative colitis in patients with indeterminate inflammatory bowel disease. If the prevalence of ASCA is low in intestinal tuberculosis, it is possible that ASCA can be used as serological marker to help differentiate Crohn's disease from intestinal tuberculosis.

Anti *Saccharomyces cerevisiae* Antibodies (ASCA) are directed against cell wall oligo mannosidic epitopes of *S.cerevisiae*. The test is commercially available and is an ELISA based test which can be done in most laboratories.

Therefore the aim of the present study was to assess prevalence of ASCA in CD and ITB and evaluate the role of this serological marker in differentiating these 2 diseases.

# AIMS

- To prospectively study the prevalence of ASCA among patients with Crohn's disease and intestinal tuberculosis and assess the ability of ASCA to differentiate Crohn's disease from Intestinal tuberculosis

- To assess the ability of clinical presentation, radiology, endoscopy and histopathology to differentiate Crohn's disease from intestinal tuberculosis

# *Review of Literature*

Crohn's disease(CD) and intestinal TB (ITB) are both chronic granulomatous conditions which affect the gastrointestinal tract in a similar manner and share clinical, enodscopic, radiological and histological features. Thus, often it may be difficult to differentiate between the two. Establishment of correct diagnosis is essential because the treatment and prognosis of the two diseases are entirely different.

Once considered rare in the developing world the epidemiology of IBD is changing and the incidence of both CD and ulcerative colitis (UC) is increasing in the Asian Pacific region, India, Eastern Europe and South Africa.[1-4, 5]

## Pathogenesis and genetics:

*Mycobacterium tuberculosis* is the causative organism in ITB whereas the etiology of CD is multi-factorial and includes genetic, immunological, environmental and microbial factors. Both trigger potent adaptive TH1 cytokine responses which result in granuloma formation and are characterized by robust production of interferon-gamma (IFN-γ), IL-12 and IL-23.

Current evidence suggests that CD may result from abnormal immune response to commensal gut bacteria in genetically predisposed patients [6] . Gut Inflammation is kept

in check through an active process of immune tolerance. Two specific populations of T cells, T regulatory 1 and T helper 3 (Th3) cells, appear to have similar roles in maintaining mucosal tolerance in the intestine [7]. Failure of immune tolerance and over responsiveness of gut immune system to commensal microbes may lead to CD [8]. The immune response is skewed toward cell-mediated immunity. Th1 responses support cell-mediated immunity and a delayed hypersensitivity-type response [9]. Th1 response leads to elaboration of a variety of injurious and proinflammatory substances (prostaglandins, reactive oxygen metabolites, nitric oxide, leukotrienes, and proteases) that are the ultimate cause of tissue destruction and granuloma formation. When the mucosal immune system in patients predisposed to the development of Crohn's disease is first exposed to an initiating antigenic stimulus, a dysregulated and overly aggressive cytokine-mediated T-cell response is mounted. Cytokines involved in innate immune responses, such as tumor necrosis factor α(TNF-α), interleukin-1, interleukin-6, and possibly interleukins 12 and 18, may play a key role in this phase. The antigens that perpetuate the inflammatory response are taken up by macrophages. Degradation of antigen within proteosomes in macrophages results in presentation of an epitope in the context of the class II major histocompatibility complex (MHC). Interaction between MHC class II and the T cell receptor (CD3) results in antigen-specific interaction between the macrophage and the $CD4^+$ T cell. This event is necessary to activate the T cell. Once CD4+ T cells are activated, effector cytokines involved in the adaptive immune response, including TNF-α and interferon-γ, as well as interleukins 4 and 13, mediate the effector phase of the intestinal inflammatory response. Novel cytokines such as TL1A and interleukins 23, 27, and 31 may also contribute to the effector phase[10].

Expression of adhesion molecules is critical to amplify the immune response, because the resident populations of granulocytes and mononuclear cells alone do not account for the vigorous inflammatory reaction characterizing IBD. Adhesion molecules on the leukocyte surface and their ligands on the endothelium of venules in the lamina propria interact in a coordinated multistep process that permits trafficking of inflammatory cells into the mucosa.

The sustained nature of the immune response in CD may have various causes. Poor intestinal barrier function may permit continued exposure of lamina propria lymphocytes to antigenic stimuli from the lumen. Poor barrier function also may be a factor in the onset of Crohn's disease, because such patients have increased intestinal permeability preceding clinical relapse of disease[11].Alternatively, a sustained exaggerated inflammatory reaction may result from an ineffective immune response—resulting from a variety of defects—to an ever-present stimulus, as occurs in a number of conditions in humans in which there is a known immunologic defect.. Finally, the sustained nature of the inflammation may result from a programmed over-responsiveness to a persistent stimulus. Consistent with this theory is the finding that the mucosal T cells in patients with Crohn's disease have defective apoptosis[12]. This finding could account for the sustained nature of inflammation in IBD, because programmed cell death of lymphocytes is a normal mechanism for dampening immune response.

Mycobacterium tuberculosis is the pathogen responsible for most cases of intestinal tuberculosis, but rarely it may be caused by *M. bovis*, an organism found in dairy

products,. The organism penetrates the mucosa and stimulates Th1 type of immune response which leads to tissue destruction and granuloma formation in similar fashion as in Crohn's disease. This leads to endoscopic and histologic similarities between the two disorders.

CD is a complex genetic disorder. The relative risk among first-degree relatives is 14 to 15 times higher than that of the general population which supports genetic predisposition [13]. The concordance rate among monozygotic twins is as high as 67% for Crohn's disease [14].

The presence of a locus on chromosome 16 (the so-called IBD1 locus) had been confirmed repeatedly to be linked to Crohn's disease, indicating the presence of a Crohn's disease gene in this region [15]. Two independent groups have identified the IBD1 locus as theNOD2 gene [16], [17]. NOD2 mediates the innate immune response to microbial pathogens, leading to activation of NF-κB. Persons with allelic variants on both chromosomes have a 40-fold relative risk of Crohn's disease compared to those without variantNOD2 genes[17] . A locus on chromosome 12 (IBD2) has been observed less consistently, in both Crohn's disease. Other loci suggested having linkage to IBD include regions on chromosomes 1p, 1q, 3p, 3q, 6p, and 7.

In contrast to CD the contribution of genetic mutations in intestinal TB has not been explored.

## Pathology – ITB:

Any part of GI tract can be involved but in three-fourth of cases ileocecal region is involved. Both sides of the ileocecal valve are usually involved, leading to incompetence of the valve, a finding that distinguishes tuberculosis from Crohn's disease. The other locations of involvement, in order of frequency, are the ascending colon, jejunum, sigmoi colon, rectum, duodenum, stomach and esophagus.

Three broad categories of gross appearances have been recognized [18], [19]

(1)Ulcerative lesions are seen in 60% of patients.

(2) Hypertrophic lesions occur in 10% of patients. The condition consists of scarring, fibrosis, and heaped-up mass lesions that mimic carcinoma.

(3) Ulcerohypertrophic lesions are seen in 30% of patients

The bowel wall appears thickened, and there is an inflammatory mass surrounding the ileocecal region. Strictures and even fistula may be seen. The serosal surface is covered with multiple tubercles. The mesenteric lymph nodes frequently are enlarged and thickened. The mucosa is hyperemic, cobblestoned, edematous, and, in some cases, ulcerated. In contrast to Crohn's disease, the superficial ulcers tend to be circumferential, with the long axis perpendicular to the lumen. When these ulcers heal, the associated fibrosis causes stricture and stenosis of the lumen.

Histology reveals granuloma and caseation is not commonly seen in intestinal wall where as they found with regularity in regional lymph nodes. The muscularis usually is spared. Acid-fast bacilli may be seen in tissue sections and sometimes organism may be recovered in culture of the involved tissues.

## Pathology – CD:

Most common location of disease is in the ileocecal region which is seen in upto half the cases. One third of patients have disease confined only to ileum and 25% with disease restricted to the colon [20].

Linear or serpiginous ulcers may form when multiple ulcers fuse in a longitudinal direction. With transverse coalescence of ulcers, the classic cobblestoned appearance may arise, representing a network of ulcers surrounding relatively normal mucosa and prominent submucosal edema.

Large ulcers, sinus tracts, and strictures are late features of Crohn's disease. As a result of the chronicity of the inflammatory process, free perforation is much less common than walled-off or contained intra-abdominal abscesses or fistulas to bowel, skin, bladder, or vagina. Fibrosis is another transmural aspect of the disease. Creeping mesenteric fat on serosa of bowel is highly suggestive of CD.[21]

The earliest lesion characteristic of Crohn's disease is the aphthous ulcer. The presence of granulomas is not unique in CD and is not seen in every case. Prevalence of granulomas in Crohn's disease have varied greatly, ranging from 15% in endoscopic series [22] to as high as 70% in surgical series [23]. The granulomas are usually sparse, scattered, and not well formed. In contrast to the granulomas of tuberculosis, little or no central necrosis is present, and acid-fast stains and mycobacterial cultures are negative. Focal intestinal inflammation is the hallmark pathologic finding in Crohn's disease. - focal crypt inflammation, focal areas of marked chronic inflammation, the presence of aphthae and ulcers on a background of little or no chronic inflammation, and the interspersing of segments of involved bowel with segments of uninvolved bowel. Transmural involvement is observed less commonly than disease of the mucosa and submucosa, but to the extent that transmural disease is noted, it is highly consistent with a diagnosis of Crohn's disease. The presence of lymphoid aggregates in both the submucosa and external to the muscularis propria are an accurate sign of Crohn's disease even when granulomas are not seen.

## Clinical Features:

**ITB:** The clinical features are non specific. Abdominal pain is the predominant symptom seen in upto 90% of cases. Other symptoms are weight loss, fever, diarrhea or constipation, and blood in the stool [24]. Right iliac fossa mass may be present in some cases. Malabsorption can occur when obstruction leads to proximal bacterial overgrowth.

Anemia, hypoalbuminemia and raised ESR may be the non specific laboratory abnormalities.

**CD:** Clinical presentation depends on disease location with small bowel disease usually presenting as intestinal obstruction [25]. Colonic disease typically presents as diarrhea which is sometimes blood mixed. Abdominal pain is a more frequent and persistent complaint. Pain may be intermittent and colicky in nature or sustained and severe. Constitutional symptoms, particularly weight loss and fever, or growth retardation in children may also be prominent

Perianal skin lesions include maceration, superficial ulcers, and abscesses. Anal canal lesions include fissures, ulcers, and stenosis. The anal fissures of Crohn's disease tend to be placed more eccentrically than idiopathic fissures, which tend to occur along the midline Deeper abscesses may arise secondary to fistulas, especially when the internal os is located high in the rectum.

Perianal fistulas are common, estimated to occur in 15% to 35% of patients.
Fistulas from one segment of the gastrointestinal tract to another also occur frequently. Enteroenteric, enterocolonic, and colocolonic fistulas are often asymptomatic. Fistula to the vagina may occur with penetration from a severely inflamed rectal vault anteriorly or from the small bowel. Enterovaginal fistulas tend to occur among women who have had a hysterectomy, permitting direct extension to the adjacent vaginal cuff. Patients present with foul, persistent vaginal discharge and occasionally with passage of flatus or frank stool per vagina. Enterovesicular or colovesicular fistulas may present as recurrent polymicrobial urinary tract infection or as frank pneumaturia and fecaluria. These fistulas

are notoriously difficult to heal by nonsurgical means. Enterocutaneous fistulas to the anterior abdomen, often occurring after surgery, may be especially troublesome.

Stricture is another characteristic complication of Crohn's disease. Symptoms may include colicky postprandial abdominal pain and bloating, punctuated by more severe episodes, and often culminating in complete obstruction.

## Diagnosis – ITB

Histopathology may establish the definitive diagnosis of ITB if AFB can be seen in tissue section or necrotizing granuloma is seen. AFB recovered from tissue culture can also confirm the diagnosis. Colonoscopic findings, are nonspecific, consist of superficial areas of ulceration and a nodular friable mucosa, mass lesion, stricturing[26] .

Radiographic examination of the bowel reveals a thickened mucosa with distortion of the mucosal folds, ulcerations, varying degrees of thickening and stenosis of the bowel, and pseudopolyp formation [27] [28] . Computed tomography may show preferential thickening of the ileocecal valve and medial wall of the cecum, extension to the terminal ileum, and massive lymphadenopathy with central necrosis. The cecum is contracted with disease on both sides of the valve, and the valve itself is often distorted and incompetent. Tuberculosis tends to involve short segments of the intestine with stenosis and fistula formation. In the hypertrophic form a mass can be seen that resembles a cecal carcinoma. Calcified mesenteric lymph nodes and an abnormal chest x-ray are other signs that aid in the diagnosis of intestinal tuberculosis.

## Diagnosis – CD

The diagnosis is established through a total assessment of the clinical presentation with confirmatory evidence from radiographic, endoscopic, and in most cases, pathologic findings. Anemia, hypoalbuminamia, an elevated C-reactive protein or erythrocyte sedimentation rate, although not specific for IBD, may prompt further investigation.

A small bowel follow-through study is the primary modality to evaluate for small bowel disease - Early findings include aphthous ulcers, a coarse villous pattern of the mucosa, and thickened folds. Submucosal edema may be evident as thickening or flattening of the valvulae conniventes, whereas transmural edema manifests as widening of the separation between loops of bowel. Ulcers most often occur on the mesenteric border with consequent pseudosacculation of the antimesenteric border because of shortening of the mesenteric portion[29] Later findings include a cobblestone appearance resulting from edema in relatively spared islands of mucosa that are separated by longitudinal and transverse knife-like clefts of ulceration[29]. Still later, one may discern fistulas, sinus tracts, and fixed strictures.

Computed tomography (CT) - mesenteric lymphadenopathy and transmural thickening of the bowel are usually seen. Fibrofatty proliferation of the mesentery, intra-abdominal and retroperitoneal abscess is other features.

Magnetic resonance imaging may provide visualization of complex perianal fistulas superior to pelvic CT [30].

Endoscopy - aphthous ulcers, mucosal edema, cobblestoning and discontinuous segmental nature of the disease may be seen [31].

Histopathology shows granuloma in most cases - they are usually sparse, scattered, not well formed with little or no central necrosis, and acid-fast stains and mycobacterial cultures are negative. Other features include focal crypt inflammation, focal areas of marked chronic inflammation, the presence of aphthae and ulcers on a background of little or no chronic inflammation, and the interspersing of segments of involved bowel with segments of uninvolved bowel. Transmural involvement and presence of lymphoid aggregates in both the submucosa and external to the muscularis propria are other suggestive features on biopsy.

## Differentiating Crohn's Disease from ITB

Distinguishing Crohn's disease from intestinal tuberculosis in endemic areas is challenging as both conditions have overlapping clinical, radiological, endoscopic and histological characteristics.

In the developed world IBD diagnosis is less challenging due to low prevalence of chronic enteric infections. In contrast, IBD in the developing world is overshadowed by

enteric infections as well as resource constraints in utilizing technology, making the diagnosis of IBD difficult and presumptive diagnosis of infection common.

There exists a multifaceted relationship between Crohn's disease (CD) and intestinal tuberculosis (ITB). They share common pathogenic and clinical characteristics and were thought to be one and the same disease till early twentieth century.

Despite their morphological and immunopathogenic similarities, the natural history of these two conditions is divergent. ITB is associated with significant morbidity and mortality but can be cured with a 6-month course of anti-tuberculous chemotherapy [32] [33]. By contrast, CD is a chronic condition that tends to progress with time and may require lifelong therapy to maintain disease remission in the majority of patients. In areas of high-TB prevalence, empiric treatment for TB with careful clinical review is often resorted to when diagnostic uncertainty exists. This approach is problematic as it may delay treatment for CD or make it difficult to confirm or refute a diagnosis of ITB at a later stage. Furthermore, severe adverse drug reactions to anti-tuberculous chemotherapy can complicate management with empiric therapy. Conversely treatment for CD may be disastrous if a diagnosis of intestinal TB is missed.

**Clinical Features:** Both conditions are characterized by anorexia, loss of weight, abdominal pain, altered bowel habits, rectal bleeding or the presence of an abdominal mass. More acute presentations with intestinal perforation or obstruction, or intra-abdominal abscess can also occur. One study from our institute showed that presence of significant loss of weight in an Indian patient with clinically suspected enteropathic arthropathy is an independent predictor of CD[34].

The site of involvement is also similar with a predilection for the ileo-caecal region but both can involve the gastrointestinal tract from the mouth to anus. Fever is seen in both CD and ITB. TB involvement of the lower limb joints, skin, eye and liver may mimic extra-intestinal CD. Immunologically mediated reactive polyarthritis (Poncet's disease), erythema nodosum, erythema induratum and uveitis can also be interpreted as manifestations of Crohn's [35] [36] [37]. An association between inflammation and a hypercoagulable state is common to both Crohn's and tuberculosis. Patients with IBD are at a 3.6-fold increased risk of thromboembolic disease. Similarly, patients with active TB are at risk of deep vein thrombosis [38], [39], [40].

Fistulization is one of the clinical hallmarks of CD. However, entero-enteric, entero-cutaneous and peri-anal fistulas are all well described in intestinal TB[41] [42] [43]. In a South African series, 17% of peri-anal fistulas referred to a surgical department were tuberculous in origin [44] In a series from Taiwan, 8% of ITB patients presented with anorectal disease[45].

**Routine laboratory tests and tuberculin skin testing:** The use of tuberculin skin testing (TST) as a diagnostic tool in patients with ileo-colonic inflammation has limitations. Cross reactivity with BCG, a high prevalence of environmental mycobacteria and widespread latent M. tuberculosis infection makes interpretation of a positive TST difficult. Both Crohn's disease and ITB are associated with anaemia, leukocytosis, thrombocytosis, a low serum albumin and raised inflammatory markers. Routine blood tests play no role in the differentiation of CD from ITB.

**Endoscopy:** Colonoscopy with intubation of the terminal ileum combined with endoscopic mucosal biopsy is required in the evaluation of any patient with suspected CD or intestinal TB.

The majority of ITB cases will involve the ileo-caecum with varying degrees of contiguous colon and small bowel involvement. In approximately 20% of cases, segmental colonic involvement occurs in the absence of ileo-caecal involvement and lesions in greater than two colonic sites, so-called skip lesions, may occur in up to 44% of patients[46], [47], [26], [48]. Isolated small intestinal or upper gastrointestinal tract disease is also well described[49], [41].

The type of lesion rather than the distribution has become important in differentiating CD from ITB. Lee and colleagues in the first systematic prospective analysis evaluated endoscopic findings in 44 patients with ITB and 44 patients with CD. A scoring system comprising four endoscopic features of CD (anorectal lesions, longitudinal ulcers, apthous ulcers, cobblestone appearance) vs. four endoscopic features of ITB (transverse ulcers, pseudopolyps, involvement of fewer than four segments and a patulous ileo-caecal valve) was used. With this method, a positive predictive value for CD of 94.9% and 88.9% for ITB was achieved[50]. Table 1 shows comparison of endoscopic findings between CD and ITB patients.

**Table 1. Endoscopic Features of ITB and Crohn's Disease**

| Intestinal TB | Crohn's disease |
|---|---|
| Ulcers have circumferential orientation | Longitudinal orientation |

| | |
|---|---|
| Surrounding mucosa inflamed/nodular | Surrounding mucosa normal |
| Apthous ulcers uncommon | Apthous ulcers common |
| Hyperaemic nodules-isolated or in clusters | Cobblestoning |
| Pseudopolyps | Multiple skip lesions |
| Hypertrophic mucosa | Anorectal lesions |
| Strictures | Strictures |
| Destruction of ICV and/or caecum | Preservation of ICV |

**Histopathology:** In areas endemic for M. tuberculosis, the differential diagnosis of CD and ITB poses a major challenge to pathologists as both conditions are characterized by granulomatous inflammation with overlapping histologic features. Most pathologists will be faced with this differential diagnosis in the context of endoscopic mucosal biopsies where the small sample size and superficial nature of the specimen further complicates the differential diagnosis. In ITB, the classical and pathognomonic features of caseating granulomatous inflammation and acid fast bacilli are present in <30% of cases. [26, 51, 52, 53, 54, 55, 56].

A positive TB culture has a poor yield of <20% and the diagnosis is often delayed by several weeks. This has prompted the search for additional features that may assist in differentiating ITB from CD. Retrospective studies from Southern India and South Africa

have identified a number of features that appear helpful in distinguishing CD from ITB in colonoscopic biopsies [53-55].

Apart from caseous necrosis and acid fast bacilli , features encountered exclusively, or far more frequently, in ITB include confluent granulomas, multiple granulomas in a given biopsy site, large granuloma size , bands of epithelioid histiocytes lining ulcers, submucosal granulomas and disproportionate submucosal inflammation, i.e. submucosal inflammation that significantly exceeds mucosal inflammation[53-55] .

Features seen far more frequently in CD include single granulomas and architectural distortion distant from granulomatous inflammation[54]. Prospective studies evaluating the clinical applicability of the above mentioned features in the differential diagnosis of ITB and CD are awaited with interest. The importance of taking multiple biopsies in cases of suspected ITB has been emphasized and significantly increases the diagnostic yield[54]. Biopsies should be taken from all segments of the bowel including both endoscopically normal and abnormal areas[54]. In particular, ulcerated areas should be thoroughly sampled (including multiple biopsies from both the base and the edge of the ulcer) as the diagnostic yield in ITB is highest in these lesions [57]. Table 2 shows the comparison of histological findings in patients with CD vs. ITB.

**Table 2. Prevalence of Selected Histological Parameters in Patients with Intestinal Tuberculosis (ITB) and Crohn's Disease (CD): A Comparison of Three Similar Studies (values are in %)**

| | Pulimood et al. (1999) Southern India[55] | | Pulimood et al. (2005) Southern India [54] | | Kirsch et al. (2006), Cape Town, South Africa [53] | |
|---|---|---|---|---|---|---|
| | **ITB (n = 20)** | **CD (n = 20)** | **ITB (n = 33)** | **CD (n = 31)** | **ITB (n = 18)** | **CD (n = 25)** |
| **Caseous necrosis** | 40 | 0 | 36 | 0 | 22 | 0 |
| **Confluent granulomas** | 60 | 0 | 42 | 3 | 50 | 0 |
| **≥5 granulomas/biopsy site** | 40 | 0 | 45 | 0 | 44 | 24 |
| **≥10 granulomas/biopsy site** | -- | -- | -- | -- | 33 | 0 |
| **Large granulomas** | Diameter > 200 µm | | Diameter > 400 µm | | Area > 0.05 $mm^2$ | |
| | 90 | 5 | 51 | 0 | 67 | 8 |
| **Submucosal granulomas** | 45 | 5 | 39 | 6 | 44 | 12 |
| **Ulcers lined by bands of epithelioid histiocytes** | 45 | 5 | 61 | 0 | 61 | 8 |
| **Disproportionate submucosal inflammation** | 65 | 5 | -- | -- | 67 | 10 |
| **Architectural distortion distant to granulomatous inflammation** | -- | -- | 0 | 62 | -- | -- |

.

**PCR:** Several studies suggest a role for PCR for mycobacterial DNA in the differential diagnosis of ITB and CD,[26] [51], [52], [58], [59], [60]. Four retrospective studies on formalin-fixed,

paraffin embedded colonoscopic biopsy specimens reported positive results in 22% (13/60),[51] 45% (18/40) ,[26] 64% (25/39),[52] 75% (27/36)[60] of ITB patients. Three of these also included biopsies from CD patients which were PCR was positive in 0% (0/30),[52] 0% (0/26)[60] and 5% (1/20)[51] of cases. It is interesting to note that three studies reported the PCR detection rate to be no higher in biopsies with granulomas than in those without, ,[26, 51], [52] suggesting that PCR may have utility in histologically non-diagnostic cases. A recent prospective study evaluating the role of PCR for M. tuberculosis DNA in faecal samples reported positive PCR in close to 90% patients (16/18) with ITB compared with 0/30 controls (predominantly patients with irritable bowel syndrome).[59] Thus, the few published series to date suggest promise for PCR in the differential diagnosis of ITB and CD. Larger prospective studies from endemic areas are keenly awaited.

**Radiology:** Barium studies allow visualization of the mucosal surface and luminal diameter and are valuable in demonstrating the inflammatory and cicatrising lesions found in both CD and ITB. Earlier work focussed on the pattern of ileo-caecal involvement as a means of diagnosing ITB. Examples of these include the Fleischner sign (a thickened patulous ICV combined with a narrowed terminal ileum) and Stierlin's sign (a rapid emptying of contrast through a gaping ileo-ceacal valve into a shrunken or 'amputated' caecum)[42],[60] . A long segment of small intestinal involvement, with skip lesions and preservation of the IC valve and caecum were considered typical of CD. However, these radiological signs are non-specific for either ITB or CD and a variety of

other lesions such as ulcers, strictures, fistulas, fold thickening, mucosal nodules and bowel loop separation have been described in both conditions[43],[61] .

With contrast enhanced CT scanning and MRI-ITB findings in this region include asymmetric caecal wall thickening, an inflammatory mass centred around the caecum and enveloping the terminal ileum and small homogenous pericaecal lymph nodes [62],[27]. Features of CD include symmetrical bowel wall thickening, fibrofatty proliferation of the mesentry known as 'creeping fat', regional mesenteric nodes measuring 3-8 mm and enlarged mesenteric vascular bundles in the involved mesentry known as the comb sign [63],[64] .Extra-intestinal features of CD such as fatty liver, gallstones, primary sclerosing cholangitis and sacro-ileitis may also be seen on CT scan and MRI [63], [65].

Functional MRI with improved luminal contrast techniques and CT enteroclysis allow better fistula definition and distinction between bowel wall fibrosis and inflammation[65], [66]; however, the diagnostic capabilities of these new imaging modalities in environments with high rates for TB is unknown. At present conventional radiology in the majority of cases is not diagnostic.

**Laparoscopy:** No systematic laparoscopic study comparing ITB to CD has been conducted. Laparoscopy has been used as a diagnostic test in CD and the presence of creeping fat is associated with transmural inflammation. However, mesenteric fat

wrapping has also been described in patients undergoing laparotomy for tuberculosis in India[67], [68], [69].

.

# ASCA

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are antibodies directed primarily against a 200 kDa- phosphopeptidomannan cell wall component of the common baker's or brewers yeast' *Saccharomyces cerevisiae*[70].

ASCA positivity may demonstrate disturbed immune response to normal gut microbes. This was demonstrated from a study from Switzerland where lymphocytes from patients with CD, ulcerative colitis, and healthy controls were tested for their proliferative response after stimulation with the yeast antigen mannan. Only lymphocytes of ASCA-positive patients with CD proliferated after stimulation with mannan. Thus a disturbed humoral and cellular response to the yeast antigen mannan was specifically seen in a subgroup of CD patients[71]. The proposed mechanism of ASCA formation was leakiness of gut barrier but evidences are contradictory. In a study from Austria intestinal permeability was measured by lactulose/mannitol test and ASCA was quantified by using ELISA. Elevated serum levels of anti-S. cerevisiae antibodies did not seem to result primarily from a defect of the gut barrier[72].

The prevalence of ASCA (**Table 3**) has varied from 30% to 70% in various studies with studies from west reporting a higher prevalence [73], [74],[75]. Evidence suggests that these antibodies may predict the development of IBD years before the disease occurs[76]. ASCA may also be seen in 1 to 7% of normal healthy subjects [77], [78]. Sensitivity of ASCA testing ranges from 41%-76%, PPV 88% and NPV 68%[79]. ASCA is also seen in first

degree relatives of patients with CD with a higher frequency than normal and may predict development of CD in future[80],[81]. Also higher concordance of ASCA in monozygotic twins indicate genetic predisposition to develop ASCA antibody[82]. ASCA IgG and IgA levels in CD patients are highly variable[83],

**Table 3: Prevalence studies of ASCA in CD**

| | **Number of patients** | **Prevalence %** |
|---|---|---|
| Desplat-Jégo S[73] (France) | 109 | 37.9% |
| Kaila B[74] (Canada) | 114 | 34.2% |
| Hisabe T[75] (Japan ) | 68 | 45.6% |
| Peeters M[78](Belgium) | 407 | 59.7% |
| Seibold F[84] (Germany) | 71 | 68% |
| Makharia [85] (India) | 59 | 50.8% |

**ASCA: CD vs. ITB**

Two studies have been done in India to evaluate whether ASCA can distinguish Crohn's disease from intestinal tuberculosis[85], [86] . Both studies failed to demonstrate usefulness of ASCA in this regards. First study was one in New Delhi where prevalence of ASCA IgG was 50.8% and 46.6% in patients with CD and ITB respectively. Second study was one in Lucknow where prevalence of ASCA was 62% and 50% in patients with CD and ITB respectively. Both the studies did not show a significant difference in ASCA prevalence

between ITB and CD group. Thus available evidence does not support a role of ASCA in distinguishing CD from ITB.

**ASCA: Phenotype and Progression**

Apart from playing a diagnostic role in CD, ASCA has been shown to correlate with disease phenotype as well as clinical course. Kim et al studied 115 patients with Crohn's disease, diagnosed and treated between 1990 and 2004 in Korea and found the anti-*S. cerevisiae* antibody prevalence in 38.3% patients[87]. There was no difference in anti-*S. cerevisiae* antibody expression between genders. The mean age at diagnosis was younger for the anti-*S. cerevisiae* antibody positive group than the negative group (25.3 years versus 29.7 years, $p<0.05$). Clinical manifestations and laboratory tests at diagnosis did not differ between the groups. The anti-*S. cerevisiae* antibody positive group had increased fibrostenosis (B2) and penetration (B3) compared to negative group. Anti-*S. cerevisiae* antibody positive patients were admitted to the hospital more frequently than anti-*S. cerevisiae* antibody negative patients ($p<0.05$). The Harvey-Bradshaw index score was higher in the anti-*S. cerevisiae* antibody positive group than in the negative group during the follow-up period ($p<0.05$). In addition, steroid (72.7% versus 52.1%, $p<0.05$) and immunosuppressive (45.5% versus 23.9%, $p<0.05$) treatments were more frequently given to the anti-*S. cerevisiae* antibody positive group.

A study by Hungarian IBD Study Group which included 558 patients with IBD found that ASCA positivity was associated with ileal involvement, noninflammatory disease behavior and NOD2/CARD15 genotype, but not with risk for surgery[88] . Another study in 117 patients from CD from US showed that ASCA+ patients had a greater frequency

of mutant NOD2/CARD15 alleles and higher ASCA titers were associated with higher probabilities of ileal CD and stricturing/penetrating behavior independently of NOD2/CARD15 status. Higher ASCA titers were associated with more rapid development of complications[89]. A study among 139 children with CD in Canada showed that ASCA positivity predicted the early occurrence of complications but not surgery in pediatric Crohn's disease patients. Another study from Italy showed that ASCA titers correlated significantly with disease activity, and children with severe active disease showed higher ASCA values compared to those in remission[90]. A study from UK in 231 CD patients showed rapid disease progression among ASCA positive group[91]. Patients with Crohn's disease who are positive for ASCA IgA, IgG, or both, may define a subset of patients with Crohn's disease at increased risk for early surgery[92]. Table 4 summarises the phenotypic and prognostic correlates of ASCA positivity in various studies.

**Table 4: ASCA positivity vs. phenotype and progression**

| | Number of patients | Comments |
|---|---|---|
| **Kim et al, [87]** | 115 | Prevalence 38.3%<br>Stenosis, fistula, hospitalisations, higher HBS score in ASCA positive group |
| **Hungarian IBD Study Group[88]** | 558 | Prevalence 59.3%<br>Ileal disease & NOD2/CARD15 mutation more in ASCA positive group |
| **Dassopoulos T[89]** | 117 | Ileal disease & NOD2/CARD15 mutation more in ASCA positive group |
| **Amre DK[93]** | 139 | Pediatric population studied<br>More early complication |
| **Smith BR[91]** | 231 | Rapid progression of disease in ASCA positive group |
| **Forcione DG[92]** | 70 | More chance of early surgery in ASCA positive group |

**Other Serological Markers of CD:**

Apart from ASCA, other serological markers have been studied in CD. One study from Czech Republic used carbohydrate assays based on oligosaccharide chitobioside carbohydrate - anti-chitobioside carbohydrate antibodies (ACCA), laminaribioside carbohydrate anti-laminaribioside carbohydrate antibodies (ALCA), and mannobioside carbohydrate - anti-mannobioside carbohydrate antibodies (AMCA). They found that ASCA was still the best serological marker for Crohn's disease.[94] Pancreatic autoantiboies were found to have a high PPV in distinguishing CD from ulcerative colitis in a study comprising of Chinese and Caucasian population[95]. Antibody to Escherichia

coli outer membrane porin (Anti OmpC) in CD has not been promising though it picks up a few cases missed by other tests[70].

**ASCA in other diseases:**

Apart from CD, ASCA has been associated with other diseases like celiac disease and Behcet's syndrome [96], [97], [98], [99], [100]. A study from Holland showed ASCA in 18% of children and 61% of adult patients with celiac disease [97]. Another study from Turkey suggested that patients with Behcet's Syndrome who have GI involvement may have higher levels of ASCA and this needs to be further studied[98]. Other conditions where ASCA may be positive include cystic fibrosis and ankylosing spondyloarthropathy [101], [102].

# Material and Methods

## Study design:

Prospective descriptive pilot study.

## Sample size:

This was a pilot study where at least 20 subjects in each group were required. We studied 30 subjects in each group (ITB and CD).

## Subjects:

Patients presenting to the outpatient or inpatient department of Christian Medical College, Vellore from January 2006 to October 2007 with confirmed diagnosis of Crohn's disease or intestinal tuberculosis were included in the study after informed consent. Thirty patients were recruited in each group. 100 healthy controls were also studied to establish the normal value of ASCA in our population as no such data was available from our country.

Diagnosis of Crohn's disease and intestinal tuberculosis were based on standard clinical, radiological, endoscopic, histological criteria and response to treatment.

## Diagnostic criteria of Crohn's Disease:

Presence of following features in varying combination

**Endoscopy:** Skip areas, cobblestone appearance, linear or serpiginous ulcer, fistula

**Histopathology:** Crypt architecture abnormalities, mononuclear infiltration, granulomas, transmural inflammation, segmental distribution of the lesion, patchy and focal inflammation.

**Radiology:** Skip lesions, fistula, cobblestone appearance, deep linear ulcer, asymmetric involvement, creeping fat.

**Response to treatment**

## Diagnostic criteria of Intestinal Tuberculosis:

Presence of any of the following features in the appropriate clinical, endoscopic or radiological profile suggestive of intestinal tuberculosis

**1. Histopathology**

a. AFB positive on histopathology or culture

b. Caseating granuloma

c. Large or confluent granuloma

**2. Response to treatment**

Clinical profile, imaging studies, enoscopy and histopathology were evaluated in both groups of patients (CD and ITB) to assess the ability of the above to differentiate Crohn's disease from Intestinal Tuberculosis

## Anti *Saccharomyces cerevisiae* Antibodies (ASCA) test

After obtaining informed consent, 5 ml of venous blood was collected. The blood samples were stored at –20ºC. Evaluation of Anti-*Sachromyces cerevisiae* mannan antibodies (ASCA) was done at Wellcome Microbiology laboratory, C.M.C., Vellore, using the IgG ASCA ELISA kits (AIDA diagnostics, Germany). Each kit has 96 wells and a maximum of 90 samples could be processed (remaining 6 needed for controls and calibrators).

## Principle of the test:

ASCA –Ig G test is a solid phase enzyme immunoassay (ELISA) employing highly purified mannan for the detection of Ig G anti-*Saccharomyces cervisiae* antibodies in human serum. ASCA recognises specific mannan, a component of the outer wall of yeast. Serum samples diluted to 1:101 are incubated in the microplates coated with specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in subsequent steps. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the

antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off.

Addition of 3,5,3',5'-tetramethylbenzidine (TMB)-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (stop solution) and colour changes to yellow. The rate of colour formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

## Assay procedure:

Prior to pipetting, concentrated reagents were diluted. Concentrated sample buffer was diluted to 1:5 with distilled water. Concentrated wash buffer was diluted to 1:50 with distilled water and 200ml of diluted wash buffer was prepared. Serum samples were diluted to 1:101 with sample buffer and they were mixed well. 300 micro litre of diluted wash buffer was pipetted into each of the 96 wells and left for 20 seconds after which after it was cleared. This process was repeated twice.

100 micro litre of each patient's diluted serum was pipetted into designated microwells. 100 micro litres of calibrators, negative and positive controls were pipetted into the designated wells. The microwells were incubated at room temperature for 30 minutes. The microwells were then washed thrice with diluted washing buffer. Next, 100 micro litre of conjugate was pippeted into each well which was incubated at room temperature for 15 minutes. Again, the microwells were washed thrice with diluted washing buffer.

100 micro litre of TMB substrate was pipetted into each well which was incubated at room temperature for 15 minutes in dark. Finally 100 microlitre of stop solution was pipetted into each well and was incubated at room temperature for 5 minutes. The absorbance was read at 450 nm within 30 minutes.

The optical density (OD) of each calibrator (y axis) was plotted against the corresponding concentration values in U/ml (x axis). From this plot the antibody concentration of each patient and control sample was calculated by finding out the concentarion corresponding to their respective OD values.

# Statistical Methods

Average value was denoted as mean with standard deviation for normally distributed continuous variables and as median with interquartile range for non-normally distributed continuous variables. The results between ITB and CD groups were compared for statistically significant difference. For categorical variables (including ASCA result) chi square test was used. For continuous variable with normal distribution, t test was used. For continuous variable with non-normal distribution, Mann-Whitney's U test was used.

p value of <0.5 was considered significant. The statistical analysis was done using SPSS software for windows version 11.

# *Results*

Demographic features in patients with CD and ITB are shown in Table 1. There was no significant difference in age, sex and geographical location in patients with CD and ITB. Figure 1 shows the sex and state wise distribution of patients. In ITB group, 18 patients were from east India, 10 from south India and 2 from central India. In CD group 20 patients were from east India, 9 from south India and 1 from central India.

**Table 1: Demographic features**

| | **CD (n=30)** | **ITB (n=30)** | **p** |
|---|---|---|---|
| **Age (yrs.)** | 33.9 $\pm$ 15.2 | 35.1 $\pm$ 12.2 | 0.72 |
| **Sex (M/F)** | 21/9 | 16/14 | 0.29 |
| **Geographic distribution (East/South/Central)** | 20/9/1 | 18/10/2 | 0.78 |

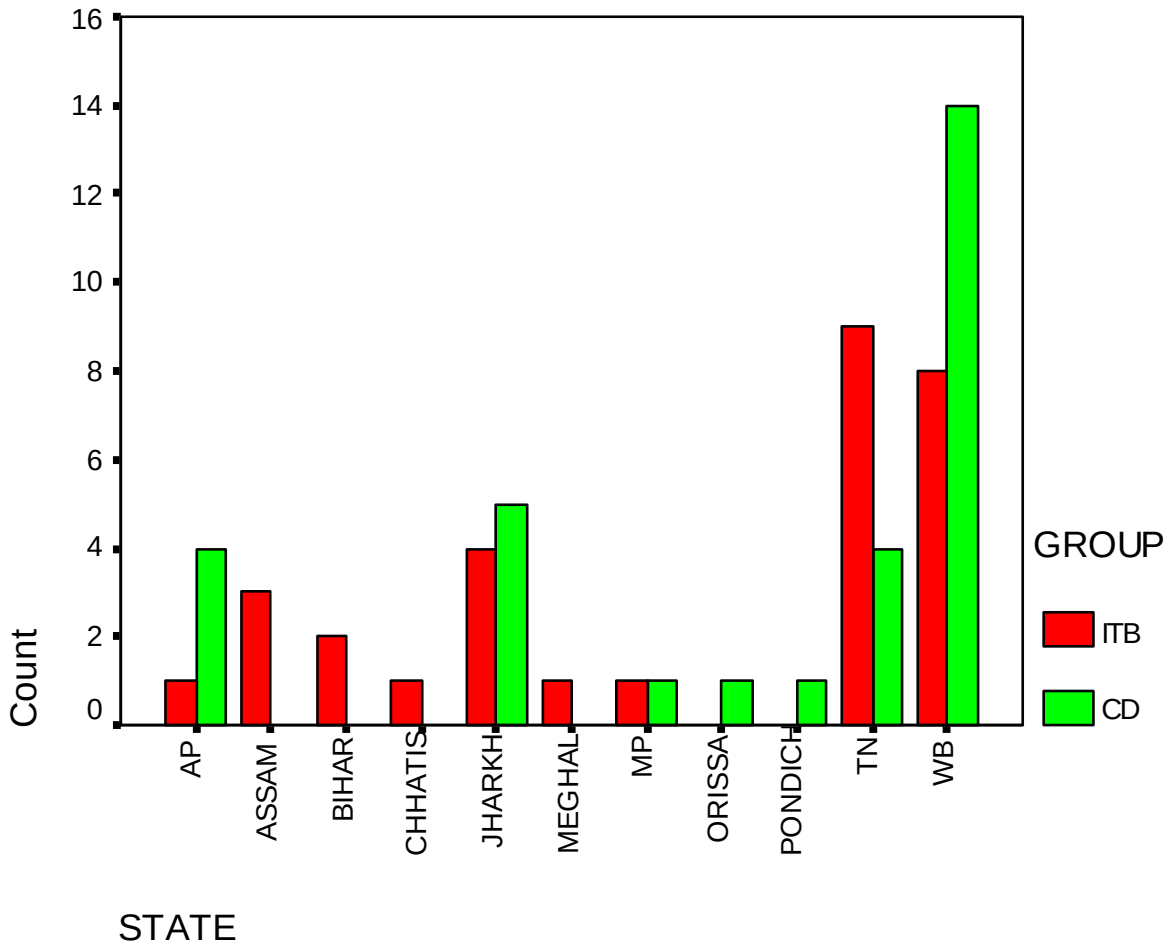


**Figure 1:** Geographic distribution of patients with CD and ITB

## Clinical Profile:

**Symptoms:**

The median duration of symptoms in patients with ITB was 3 months (range 1 month to 2 years) while it was 2 years (range 2 months to 15 years) in patients with CD, the difference being statistically significant ($p < 0.001$, Mann Whitney test).

Clinical presentations of patients with ITB and CD are shown in Table 2. Dull aching and poorly localised abdominal pain was the commonest symptom in patients with CD (60%) and ITB (70%). Diarrhoea and fever were similar in both groups. Blood mixed with stool was seen more often in patients with CD (p=0.006). Anorexia (P=0.008) and weight loss (P=0.067) were seen more often in ITB. Clinical Examination of abdomen showed right iliac fossa mass in two patients with CD. In patients with ITB right iliac fossa mass was revealed in two patients, hepatosplenomegaly in one, hepatomegaly in one and cervical lymphadenopathy in two patients.

**Table 2: Clinical features**

| | **CD ( n=30)** | **ITB ( n=30)** | **p** |
|---|---|---|---|
| **Abdominal pain** | 18(60%) | 21(70%) | 0.59 |
| **Diarrhea** | 14(46.7%) | 12(40%) | 0.8 |
| **Weight loss** | 13(43.3%) | 21(70%) | 0.067 |
| **Anorexia** | 12(40%) | 23(76.7%) | **0.008** |
| **Blood in stool** | 11(33.3%) | 1(3.3%) | **0.006** |
| **Fever** | 8(26.7%) | 9(30%) | 1.0 |

## Complications:

Intestinal and extraintestinal complications in patients with CD and ITB are shown in Table 3

**Crohn's disease:** Two patients had internal fistula (jejunocolic and colovesical), 4 had fistula in ano and two had anal fissure. Extraintestinal manifestations were present in 4 patients – 3 had arthralgia, 1 had pyoderma gangrenosum and 3 had oral ulcers alone. Thirteen patients underwent surgery for their disease – 5 had small bowel surgery for strictures, 7 had right hemicolectomy for ileocolonic disease and 1 had fistulectomy for perianal fistula.

**Intestinal Tuberculosis:** Two patients had internal fistula ( duodenocolic and jejunocolic) and 1 had fistula in ano. Extraintestinal manifestation was present in only 1 patient who had arthralgia. Three patients underwent surgery for their disease – 1 had small bowel surgery for strictures and other two had partial colonic resections as treatment of internal fistulas. Results show that occurrence of fistula and extraintestinal manifestations were not significantly different between the two groups. Patients with CD underwent surgery significantly more often compared to ITB patients.

**Table 3: Complications**

| | **CD ( n=30)** | **ITB ( n=30)** | **p** |
|---|---|---|---|
| **Fistula** | 6 (20%) | 3(10%) | 0.47 |
| **Extraintestinal manifestations** | 4 (13.3%) | 1(3.3%) | 0.35 |
| **Surgery** | 14(46.6%) | 3(10%) | **0.007** |

# Investigations:

## Blood tests:

Table 4 shows comparison of laboratory tests between patients with CD and ITB. Lab investigations revealed low albumin (<3.5 g/dl in 38 patients), high ESR (>30 in 40 patients) and borderline low haemoglobin (<11.0g% in 32 patients) in most patients. ESR was significantly higher in ITB group compared to CD (p=0.05).

**Table 4: Laboratory tests**

| Test | CD | ITB | p |
|---|---|---|---|
| **CRP(%Positive)** | 71.4 (n=28) | 75 (n=12) | 1.0 |
| **Hb(g%)** | 11.0 (n=30) | 10.5 (n=30) | 0.33 |
| **ESR(mm/hr.)** | 44.1 (n=30) | 62.8 (n=25) | **0.05** |
| **Albumin (g/dl)** | 3.3 (n=30) | 3.0 (n=29) | 0.19 |

# Endoscopy:

Colonoscopic finding in patients with CD and ITB are shown in Table 5. Colonoscopy was performed for all patients with CD and 24 patients with ITB. Twenty five (83%) patients with CD had colonic involvement. Mucosal erythema, loss of vascular pattern, ulcerations and friability were common abnormalities seen. Eleven patients had aphthous

ulcers and skip lesions , nine patients had pseuopolyps and 3 had cobblestone pattern . Eight patients had colonic strictures (5 in transverse colon, 2 in sigmoid colon and 1 in rectum) and ileum could not be visualized in these patients.

Twenty three patients with ITB(95.8%) had colonic involvement. Contiguous involvement of mucosa, ulceronodular lesions, ulcerations and friability were common abnormalities detected. Pseudopolyps were seen in two patients and two had skip lesions. Terminal ileum could not be visualized in 9 patients - four patients had colonic strictures ( 2 in ascending colon and 2 in transverse colon) while in five patients the IC valve was narrowed and deformed.

**Table 5 : Colonoscopic findings in patients with TB and CD**

| **Findings** | **CD (n=30)** | **ITB(n=24)** | **p** |
|---|---|---|---|
| **Aphthous ulcer** | 11(36.3%) | 4(16.7%) | 0.13 |
| **Skip lesions** | 11(36.3%) | 2(8.3%) | **0.02** |
| **Cobblestone pattern** | 3(10%) | 0(0%) | 0.25 |
| **Pseudopolyp** | 9(30%) | 2(8.3%) | 0.09 |
| **Stricture** | 8(26.6%) | 4(16.7%) | 0.51 |
| **Narrowed IC valve** | 3(10%) | 5(20.8%) | 0.44 |
| **Patulous IC valve** | 2(6.6%) | 2(8.3%) | 1.00 |

Apart from the presence of skip lesions (p=0.02), other colonoscopic findings were not helpful in differentiating CD from ITB.

Five patients with CD had UGI involvement on enoscopy and histology (4 in stomach and 1 in duodenum) .

One patient had intraoperative enteroscopy which revealed multiple ileal strictures.

## Radiology:

Radiological tests included ultrasonogram of abdomen, CT abdomen and barium meal follow through(BAMFT). These tests were individualized according to patient's indications and physician preference.

**Crohn's disease:** Twelve patients had BAMFT and seven had CT abdomen. Eleven patients had abnormalities on BAMFT – 7 patients had both large and small bowel abnormalities whereas 4 patients had only small bowel abnormalities. Abnormalities included stricturing, ulceration, mucosal nodularity/edema and loss of mucosal folds. All seven patients who had CT abdomen showed abnormal finings which included bowel wall thickening, deformity, luminal narrowing and mesenteric adenopathy. Ultrasonogram of abdomen was abnormal in 2 patients and both had right colonic wall thickening.

**Intestinal tuberculosis:** Seven patients had BAMFT and six patients had CT abdomen. Six patients had abnormalities on BAMFT – 5 patients had both large and small bowel abnormalities whereas 1 patient had only small bowel abnormalities. Abnormalities included stricturing, deformity, mucosal nodularity and ulcerations. All six patients who had CT abdomen showed abnormal findings which included bowel wall thickening, luminal narrowing, deformity and mesenteric adenopathy (similar to patients with CD). Ultrasonogram of abdomen was abnormal in 5 patients - 4 had ileocecal region bowel wall thickening while one had ileal thickening.

Table 6 compares radiological findings in patients with CD and ITB who had BAMFT and/or CT abdomen. Contracted cecum, narrowed IC valve and mesenteric adenopathy was significantly commoner in patients with ITB.

**Table 6: Comparison of radiological finings of CD and ITB patients**

| Findings | CD(n=19) | ITB(n=13) | p |
|---|---|---|---|
| **Contracted cecum** | 1(5.3%) | 8(61.5%) | **<0.001** |
| **Narrow IC vave** | 1(5.3%) | 5(38.5%) | **0.03** |
| **Patulous IC valve** | 1(5.3%) | 0(0%) | 1.0 |
| **Colonic stricture** | 0(0%) | 2(15.4%) | 0.16 |
| **Ileal stricture** | 5(26.3%) | 1(7.7%) | 0.36 |
| **Multiple ileal strictures** | 4(21.1%) | 0(0%) | 0.13 |
| **Aphthous ulcer** | 2(10.6%) | 1(7.7%) | 1.0 |
| **Pseudopolyp** | 0(0%) | 0(0%) | 1.0 |
| **Cobblestone pattern** | 1 | 1 | 1.0 |
| **Mesenteric LN** | 2 | 7 | **0.02** |
| **Fistula** | 2 | 2 | 1.0 |

# Histopathology:

Histological features suggestive of CD were seen 90% of CD patients and these included crypt architecture abnormalities, mononuclear infiltration, granulomas, transmural inflammation, segmental distribution of the lesion and patchy and focal inflammation. Remaining three patients had non-specific histological features and in them, clinical and radiological features along with response to therapy formed the basis of diagnosis.

Histological features suggestive of ITB was seen in 70% of ITB patients and these included caseating granuloma, large or confluent granuloma and presence of AFB in tissue section (or culture of tissue). In the remaining patients clinical and radiological features along with response to therapy formed the basis of diagnosis as was the case with 3 patients with CD. Table 7 shows the comparison of histological findings between the two groups.

**Table 7: Comparison of histological findings between the two groups**

| **Findings** | **CD (n=30)** | **ITB ( n=24)** | **p** |
|---|---|---|---|
| **Confluent granuloma** | 0(0%) | 9(37.5%) | **<0.001** |
| **Discrete granuloma** | 9(30%) | 11(45.8%) | 0.27 |
| **Microgranuloma** | 3(10%) | 0(0%) | 0.25 |
| **Caseous necrosis** | 0(0%) | 2(8.33%) | 0.19 |
| **AFB positive** | 0(0%) | 9(37.5%) | Not relevant |

## Site of disease

The site of intestinal involvement is shown in figure 2. Ileocolonic disease was commonest followed by colonic disease.

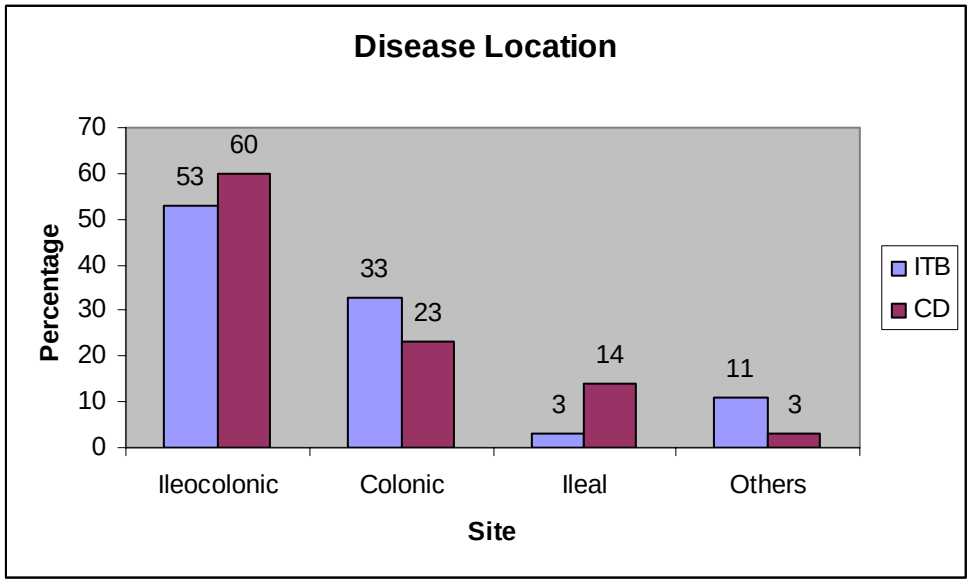


Figure 2: Disease Location

While endoscopy was able to detect colonic and terminal ileal lesions, radiology was especially helpful in patients with small bowel disease and colonic strictures (where entire colon and terminal ileum could not be visualised). Table 8 shows the site of disease picked up on radiological and endoscopic investigations. Colonic lesions were frequently missed by radiological investigations.

# Anti *Saccharomyces cervisiae* Antibody test – Results

ASCA ELISA test was done on 100 controls to establish the normal "cut-off" antibody levels in the population. The mean optical density value plus 2 standard deviation in controls was 6.9 U/ml and was taken as "cut off" value. Patients with value above 6.9U/ml were considered to have ASCA test positive.

Figure3 and Table 8 shows ASCA results of CD patient compared with ITB.

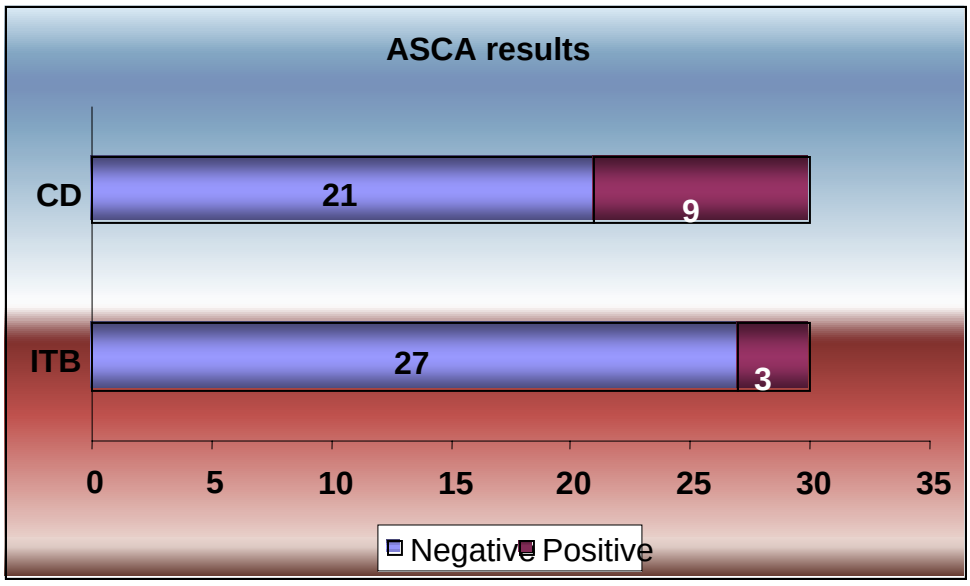


**Figure 3**

**Table 8:** ASCA results of CD patient compared with ITB

| ASCA | CD | ITB | p (Chi square) |
|---|---|---|---|
| **Positive** | 9(30%) | 3(10%) | **0.10** |
| **Negative** | 21(70%) | 27(90%) | |
| **Total** | 30 | 30 | |

30% of patients with CD had ASCA positive as compared to 10% of patients with ITB.

Table 9 compares demographic, clinical, investigation profile between ASCA positive and negative groups  in CD. No significanr difference was observed in any parameter tested.  The occurrence of stricturing and fistulising disease, small bowel disease, need for surgery, marker of disease activity (CRP, ESR, Harvey Bradshaw Score>4) and clinical course was similar in the two groups.

**Table 9: Comparison of ASCA positive and negative patients with CD**

| | ASCA positive (n=9) | ASCA negative (n=21) | p |
|---|---|---|---|
| **Sex (females, %)** | 44.4 | 28.6 | 0.43 |
| **Age (yrs.)** | 27(25) | 35(20.5) | 0.46 |
| **Region(South/East/Others)** | 22.2/66.7/11.1 | 33.3/66.7/0 | 0.26 |
| **Duration of illness (yrs.)** | 1.5(4.1) | 2(4.7) | 0.86 |
| **Abdominal pain (%)** | 77.8 | 52.4 | 0.25 |
| **Diarrhoea (%)** | 55.6 | 42.9 | 0.69 |
| **Blood in stool (%)** | 22.2 | 38.1 | 0.68 |
| **Anorexia (%)** | 33.3 | 42.9 | 0.70 |
| **Weight loss (%)** | 33.3 | 47.6 | 0.69 |
| **Fever (%)** | 33.3 | 23.8 | 0.67 |
| **Fistula (%)** | 22.2 | 19 | 1.0 |
| **Bowel Stricture (%)** | 44.4 | 57.1 | 0.69 |
| **Extra intestinal features** | 22.2 | 9.5 | 0.56 |
| **Ileal disease (%)** | 88.9 | 81 | 1.0 |
| **Hb (g %)** | 11.4(.5) | 11.3(3.8) | 0.63 |
| **ESR (mm/hr.)** | 65(52) | 33(23.5) | 0.07 |
| **CRP positive** | 77.8 | 61.9 | 0.4 |
| **Albumin (g %)** | 3.4(0.9) | 3.3(1.4) | 0.89 |
| **HBS > 4** | 11.1 | 4.8 | 0.52 |
| **Non specific biopsy (%)** | 11.1 | 9.5 | 1.0 |
| **Surgery (%)** | 33.3 | 47.6 | 0.69 |

# Discussion

The study was aimed at assessing ASCA as a serological marker to distinguish between CD and ITB. Clinical and investigation profile of the two groups were also compared to assess theirability to ifferentiate CD from ITB. Being a pilot study only 30 patients with CD and 30 with ITB were studied.

The mean age distribution between the two groups was comparable. Males predominated in the CD group while the ITB group had almost equal representation of both males and females. Most patients studied came from East and South India reflecting the patient population visiting CMC. Geographic distribution of patients was similar in CD and ITB groups.

As reported in literature abdominal pain was the commonest symptom in both groups[24]. Anorexia was significantl more common in ITB group while blood mixed stool was significantly more common in CD group. Apart from these, other symptoms were comparable between the two groups. Thus, blood mixed stool was a good indicator of CD. Extraintestinal manifestations were uncommon in both groups. Fistulising disease and extraintestinal manifestations were more often seen in CD, but the ifference was not statistically significant.

Anemia, and hypoalbuminemia were the non specific laboratory abnormalities seen in both groups. While CRP positivity did not differ between the two groups, ESR was significantly higher in the ITB groups.

Colonoscopy showed skip lesions were significantly more common in CD. Aphthous ulcers and cobblestone pattern were more common in CD as compared to ITB but the difference was not statistically significant. Patulous IC valve and pseudopolyps are reported to be commoner in ITB compared with to CD. However this was not reflected in the present study.

Radiological studies (Barium Meal Follow Through, CT Abdomen) showed that contracted cecum, narrow IC valve and mesenteric adenopathy were significantly more common in ITB patients. Other findings were similar in both groups. This observation is similar to that reported in literature where findings like strictures and fistulas, fold thickening and mucosal nodules have been described in both conditions[43], [61].

Histopathological findings in C D and ITB were similar except for confluent granuloma being more common in ITB ($p < 0.001$).

As we wanted to test the ability of ASCA to differentiated CD and ITB, confirmed cases of CD and ITB were recruited in the study. Patients with ambiguous histology and no response to therapy were not included.

ASCA positivity has varied from 30 to 70% in various series[73], [74], [75]. In our study 30% of patients with CD were ASCA positive which is on the lower end of spectrum of reported prevalence. 10% of patients with ITB were ASCA positive but the difference failed to reach statistical significance. Two other studies done in India also showed similar results of ASCA positivity in CD and ITB(Table 10). The two studies from India showed higher prevalence rates and less pronounced difference of ASCA in CD and ITB as compared to the current study (Table 10). The reason could be because we established our own 'ASCA cut off ' level by analysing serum of 100 healthy controls .

**Table 10: ASCA in differentiating CD from ITB (NS- not significant)**

| | **ASCA in CD** | **ASCA in ITB** | **p** |
|---|---|---|---|
| **Makharia et al[85]** | 50.8% | 46.6% | NS |
| **Ghoshal et al[86]** | 62% | 50% | NS |
| **Current study** | 30% | 10% | NS |

ASCA has also been correlated with phenotype and course of CD with younger age of onset, ileal disease, stricturing and fistulising disease being more common in patients with a positive serology along with a higher chance of undergoing surgery [87], [88], [89], [91], [92]. Similar findings were not seen in our study. An interesting finding in our study was that

eight of the nine patients with CD with ASCA positive had ileal involvement. Hence ASCA positivity may point to small bowel involvement in CD.

Thus ASCA has a low prevalence among CD patients in our country and is unable to differentiate CD from ITB.

# Conclusions

Differentiating CD from ITB continues to be a challenging problem. Our study confirms the results of 2 other studies from India that ASCA does not help to differentiate CD from ITB [85], [86]. Presently, a combination of clinical features, endoscopy, histology, radiology and response to treatment continues to be the mantra to differentiate these two conditions. We need to continue to strive to develop new tests to help clinicians differentiate the two conditions.

# Bibliography:


1. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel disease: up or down? World J Gastroenterol 2006;14:6102-8.
2. Ouyang Q, Tandon R, Goh K-L et al. The emergence of inflammatory bowel disease in the Asian Pacific region. Curr Opin Gastroenterol 2005;21:408-13.
3. Sharma MP, Bhatia V. Abdominal tuberculosis. Indian J Med Res Volume 120, 2004:305-15.
4. Wright JP, Froggatt J, O'Keefe EA, et al . The epidemiology of inflammatory bowel disease in Cape Town 1980-1984. S Afr Med J 1996;70:10-15.
5. Desai HG, Gupte PA. Increasing incidence of Crohn's disease in India: is it related to improved sanitation? Indian J Gastroentero 2005;24:23-24.
6. Kelly JK, LR S. The chronological sequence in the pathology of Crohn's disease. J Clin Gastroenterol 1998;10:28-33.
7. Ma Donald TT . Effector and regulatory lymphoid cells and cytokines in mucosal sites. Curr Top Microbiol Immunol 1999;236:113.
8. Duchmann R, May E, Heike M, et al. T cell specificity and cross reactivity towards enterobacteria, bacteroides, bifidobacterium, and antigens from resident intestinal flora in humans. Gut 1999;44:812.
9. Fuss IJ, Neurath M, Boirivant M, et al . Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP

cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. J Immunol 1996;157:1261.

10. Cominelli F. Cytokine-Based Therapies for Crohn's Disease — New Paradigms NEJM 2004;351:2045-2048.

11. Peeters M, Nevens H, Baert F, et al. Familial aggregation in Crohn's disease: Increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 1996;111:597

12. Sturm A, Fiocchi C. Life and death in the gut: More killing, less Crohn's. Gut 2002;50:148.

13. Binder V. Genetic epidemiology in inflammatory bowel disease. Dig Dis 1998;16:351.

14. Bayless TM, Tokayer AZ, Polito JM, et al. Crohn's disease: concordance for site and clinical type in affected family members–potential hereditary influences. Gastroenterology 1996;111:573.

15. Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996;379.

16. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature;411:603.

17. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599.

18. Horvath KD, Whelan RL. Intestinal tuberculosis: return of an old disease. Am J Gastroenterol 1998;93:692.

19. Marshall JB. Tuberculosis of the gastrointestinal tract and peritoneum. Am J Gastroenterol 1993;88:989.

20. Munkholm P. Crohn's disease—occurrence, course and prognosis. An epidemiologic cohort-study. Dan Med Bull 1997;44:287.

21. Desreumaux P, Ernst O, Geboes K, et al. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. Gastroenterology 1999;117:73.

22. Okada M, Maeda K, Yao T, et al. Minute lesions of the rectum and sigmoid colon in patients with Crohn's disease. Gastrointest Endosc 1991;37:319.

23. Chambers TJ, BC M. The granuloma in Crohn's disease. Gut 1979;20:269.

24. Shah S, Thomas V, Mathan M, et al. Colonoscopic study of 50 patients with colonic tuberculosis. Gut 1992;33:347.

25. Higgens CS, Allan RN. Crohn's disease of the distal ileum. Gut 1980;21:933.

26. Kim KM, Lee A, Choi KY, et al . Intestinal tuberculosis: clinicopathologic analysis and diagnosis by endoscopic biopsy. Am J Gastroenterol 1998;93:606-9.

27. Balthazar EJ, Gordon R, Hulnick D. Ileocecal tuberculosis: CT and radiologic evaluation. AJR Am J Roentgenol 1990;154:499.

28. Park SJ, Han JK, Kim JS, et al. Tuberculous colitis: radiologic-colonoscopic correlation. AJR Am J Roentgenol 2000;175:121.

29. Scotiniotis I, Rubesin SE, Ginsberg GG. Imaging modalities in inflammatory bowel disease. Gastroenterol Clin North Am 1999;28:391.

30. Hussain SM, Outwater EK, Joekes EC, et al. Clinical and MR imaging features of cryptoglandular and Crohn's fistulas and abscesses. Abdom Imag 2000;25:67.

31. 166. Pera A, Bellando P, Caldera D, et al. Colonoscopy in inflammatory bowel disease. Diagnostic accuracy and proposal of an endoscopic score. Gastroenterology 1987;92:181.

32. Akgun Y. Intestinal and peritoneal tuberculosis: changing trends over 10 years and a review of 80 patients. Can J Surg 2005;48:131-6.

33. Lingenfelser T, Zak J, Marks IN, et al. Abdominal tuberculosis: still a potentially lethal disease. Am J Gastroenterol 1993;5:744-50.

34. Danda D, Kurian G, Chacko A, et al. Crohn's disease in rheumatology clinic--an Indian experience. J Postgrad Med 2005;51:269-72.

35. Franco-Paredes C, Diaz-Borjon A, Senger MA, et al. . The ever expanding association between rheumatologic disease and tuberculosis. Am J Med 2006;119:470-7.

36. Umapathy KC, Begum R, Ravichandran G, et al. Comprehensive findings on clinical, bacteriological, histopathological and therapeutic aspects of cutaneous tuberculosis. Trop Med Int Health 2006;11:1521-8.

37. Kurup SK, Chan C-C. Mycobacterium related ocular inflammatory disease: diagnosis and management. Ann Acad Med Singapore 2006;35:203-9.

38. Miehsler W, Reinisch W, Valic E, et al. Is inflammatory bowel disease an independant and disease specific risk factor for thromboembolism? Gut 2004;53:542-8.

39. Robson SC, White NW, Aronson I, et al. Acute phase response and the hypercoagulable state in pulmonary tuberculosis. Brit J Haematol 1996;93:943-9.

40. White NW. Venous thrombosis and rifampicin. Lancet 1989;2:434

41. Novis BH, Bank S, Marks IN. Gastrointestinal and peritoneal tuberculosis: a study of cases at Groote Schuur Hospital S Afr Med J 1973;47:365-72.

42. Werbeloff L, Novis BH, Bank S, al e. The radiology of tuberculosis of the gastrointestinal tract. Br J Radiol 1973;46:329-36.

43. Nagi B, Sodhi KS, Kochhar R, al. e. Small bowel tuberculosis: enteroclysis findings. Abdom Imaging 2004;29:335-40.

44. Terreblanche J. Fistula in ano: a five year survey at Groote Schuur Hospital and a review of the literature. S Afr Med J 1964:403-8.

45. Chen W-S, Leu S-Y, Hsu H, et al. Trend of large bowel tuberculosis and the relation with pulmonary tuberculosis. Dis Colon Rectum 1992;35:189-92.

46. Singh V, Kumar P, Kamal J, et al. Clinicocolonoscopic profile of colonic tuberculosis. Am J Gastroenterol 1996;3:565-8.

47. Alvares JF, Devarbhavi H, Makhija P, et al. Clinical, colonoscopic, and histological profile of colonic tuberculosis in a tertiary hospital. Endoscopy 2005;37:351-6.

48. Leung VKS, Law ST, Lam CW, et al. Intestinal tuberculosis in a regional hospital in Hong Kong: a 10 year experience. . Hong Kong Med J 2006;12:264-71.

49. Gilinsky NH, Marks IN, Kottler RE, et al. Abdominal tuberculosis: a 10 year review. S Afr Med J 1983;64:849-57.

50. Lee YJ, Yang SK, Byeon JS, et al. Analysis of colonoscopic findings in the differential diagnosis between intestinal tuberculosis and Crohn's disease. Endoscopy 2006;38:592-7.

51. Amarapurkar DN, Patel ND, Amarapurkar AD, et al. Tissue polymerase chain reaction in diagnosis of intestinal tuberculosis and Crohn's disease. J Assoc Physicians India 2004;52:863-7.

52. Gan HT, Chen YQ, Ouyang Q, et al. Differentiation between intestinal tuberculosis and Crohn's disease in endoscopic biopsy specimens by polymerase chain reaction. Am J Gastroenterol 2002;97:1446-51.

53. Kirsch R, Pentecost M, Hall Pde M, et al. Role of colonoscopic biopsy in distinguishing between Crohn's disease and intestinal tuberculosis. J Clin Pathol 2006;59:840-4.

54. Pulimood AB, Peter S, Ramakrishna B, et al. Segmental colonoscopic biopsies in the differentiation of ileocolic tuberculosis from Crohn's disease. J Gastroenterol Hepatol 2005;20:688-96.

55. Pulimood AB, Ramakrishna BS, Kurian G, et al. Endoscopic mucosal biopsies are useful in distinguishing granulomatous colitis due to Crohn's disease from tuberculosis. Gut 1999;45:537-41.

56. Zhou ZY, Luo HS. Differential diagnosis between Crohn's disease and intestinal tuberculosis in China. Int J Clin Pract 2006;60:212-4.

57. Bhargava DK, Tandon HD, Chawla TC, et al. Diagnosis of ileocecal and colonic tuberculosis by colonoscopy. Gastrointest Endosc 1985;31:68-70.

58. Anand BS, Schneider FE, El-Zaatari FA, et al. Diagnosis of intestinal tuberculosis by polymerase chain reaction on endoscopic biopsy specimens. Am J Gastroenterol 1994;89:2248-9.

59. Balamurugan R, Venkataraman S, John KR, et al. PCR amplification of the IS6110 insertion element of Mycobacterium tuberculosis in fecal samples from patients with intestinal tuberculosis. J Clin Microbiol 2006;44:1884-6.

60. Vanhoenacker FM, De Backer AI, Op de Beeck, et al. Imaging of gastrointestinal and abdominal tuberculosis. Eur Radiol 2004;14:E103-15.

61. Leder RA, Low VHS. Tuberculosis of the abdomen. Radiol Clin North Am 1995;33:691-705.

62. Pereira JM, Madureira AJ, Viera A, et al. Abdominal tuberculosis: imaging features. Eur J Radiol;55:173-80.

63. Gore RM, Balthazar EJ, Ghahremani GG, et al. CT features of ulcerative colitis and Crohn's disease. Am J Roentgenol 1996;167:3-15.

64. De Backer AI, Mortelé KJ, Deeren D, et al. Abdominal tuberculous lymphadenopathy: MRI features. Eur Radiol 2005;15:2104-9.

65. MacKalski BA, Bernstein CN. New diagnostic imaging tools for inflammatory bowel disease. Gut 2006;55.

66. Ambrosinin R, Barchiesi A, Di Mizio V, et al. Inflammatory chronic disease of the colon: how to image. Eur J Radiol 2007;61:442-8.

67. Kapoor VK. Abdominal tuberculosis. Postgrad Med J 1998;74:459-67.

68. Bhansali SK. Abdominal tuberculosis: experience with 300 cases. Am J Gastroenterol 1977;67:324-37.

69. Tandon HD, Prakash A. Pathology of intestinal tuberculosis and its distinction from Crohn's disease. Gut 1972;13:260-9.

70. Zholudev A, Zurakowski D, Young W, et al. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. Am J Gastroenterol 2004;99:2235-41.

71. Konrad A, Rütten C, Flogerzi B, et al. Immune sensitization to yeast antigens in ASCA-positive patients with Crohn's disease. Inflamm Bowel Dis 2004;2004:97-105.

72. Harrer M, Reinisch W, Dejaco C, et al.. Do high serum levels of anti-Saccharomyces cerevisiae antibodies result from a leakiness of the gut barrier in Crohn's disease? Eur J Gastroenterol Hepatol 2003;15:1281-5.

73. Desplat-Jégo S, Johanet C, Escande A, et al. Update on Anti-Saccharomyces cerevisiae antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: results of a multicenter study. World J Gastroenterol 2007;13:2312-8.

74. Kaila B, Orr K, Bernstein CN. The anti-Saccharomyces cerevisiae antibody assay in a province-wide practice: accurate in identifying cases of Crohn's disease and predicting inflammatory disease. Can J Gastroenterol 2005;19:717-21.

75. Hisabe T, Matsui T, Sakurai T, et al. Anti-Saccharomyces cerevisiae antibodies in Japanese patients with inflammatory bowel disease: diagnostic accuracy and clinical value. J Gastroenterol 2003;38:121-6.

76. Israeli E, Grotto I, Gilburd B, et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut;54:1212-3.

77. Quinton JF, Sendid B, Reumaux D, et al. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998;42:788-91.

78. Peeters M, Joossens S, Vermeire S, et al. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. . Am J Gastroenterol 2001;96:730-4.

79. Vermeire S, Joossens S, Peeters M, et al Comparative study of ASCA (Anti-Saccharomyces cerevisiae antibody) assays in inflammatory bowel disease. Gastroenterology 2001;120:827-33.

80. Török HP, Glas J, Hollay HC, et al. Serum antibodies in first-degree relatives of patients with IBD: a marker of disease susceptibility? A follow-up pilot-study after 7 years. Digestion 2005;72:119-23.

81. Glas J, Török HP, Vilsmaier F, et al. Anti-saccharomyces cerevisiae antibodies in patients with inflammatory bowel disease and their first-degree relatives: potential clinical value. Digestion 2002;66:173-7.

82. Halfvarson J, Standaert-Vitse A, Järnerot G, et al. Anti-Saccharomyces cerevisiae antibodies in twins with inflammatory bowel disease. Gut 2005;54:1237-43.

83. Norman GL. Anti- Saccharomyces cerevisiae antibodies in inflammatory bowel disease. Clin Applied Immunol Rev 2001;2:45-63.

84. Seibold F, Stich O, Hufnagl R, et al. Anti-Saccharomyces cerevisiae antibodies in inflammatory bowel disease: a family study. Scand J Gastroenterol 2001;36:196-201.

85. Makharia GK, Sachdev V, Gupta R, et al. Anti-Saccharomyces cerevisiae antibody does not differentiate between Crohn's disease and intestinal tuberculosis. Dig Dis Sci 2007;52:33-9.

86. Ghoshal UC, Ghoshal U, Singh H, et al.. Anti-Saccharomyces cerevisiae antibody is not useful to differentiate between Crohn's disease and intestinal tuberculosis in India. J Postgrad Med 2007;53:166-70.

87. Kim BC, Park S, Han J, et al. Clinical significance of anti-Saccharomyces cerevisiae antibody (ASCA) in Korean patients with Crohn's disease and its relationship to the disease clinical course. Dig Liver Dis 2007;39:610-6.

88. Papp M, Altorjay I, Norman GL, et al. Seroreactivity to microbial components in Crohn's disease is associated with ileal involvement, noninflammatory disease behavior and NOD2/CARD15 genotype, but not with risk for surgery in a Hungarian cohort of IBD patients. Inflamm Bowel Dis 2007;13:984-92.

89. Dassopoulos T, Frangakis C, Cruz-Correa M, et al.. Antibodies to saccharomyces cerevisiae in Crohn's disease: higher titers are associated with a greater frequency of mutant NOD2/CARD15 alleles and with a higher probability of complicated disease. Inflamm Bowel Dis 2007;13:143-51.

90. Canani RB, Romano MT, Greco L, et al. Effects of disease activity on anti-Saccharomyces cerevisiae antibodies: implications for diagnosis and follow-up of children with Crohn's disease. Inflamm Bowel Dis 2004;10:243-9.

91. Smith BR, Arnott ID, Drummond HE, et al. Disease location, anti-Saccharomyces cerevisiae antibody, and NOD2/CARD15 genotype influence the progression of disease behavior in Crohn's disease. Inflamm Bowel Dis 2004;10:521-8.

92. Forcione DG, Rosen MJ, Kisiel JB, et al. Patients with Crohn's disease who are positive for ASCA IgA, IgG, or both, may define a subset of patients with Crohn's disease at increased risk for early surgery. Gut 2004;53:1117-22.

93. Amre DK, Lu SE, Costea F, et al. Utility of serological markers in predicting the early occurrence of complications and surgery in pediatric Crohn's disease patients. Am J Gastroenterol 2006;101:645-52.

94. Malickova K, Lukas M, Donoval R, et al. Novel anti-carbohydrate autoantibodies in patients with inflammatory bowel disease: are they useful for clinical practice? Clin Lab 2006;52:631-8.

95. Lawrance IC, Hall A, Leong R, et al. Patients with BS who have GI involvement may have higher levels of ASCA and this needs to be further studied. Inflamm Bowel Dis 2005;11:890-7.

96. Granito A ML, Muratori P, Guidi M, et al. Anti-saccharomyces cerevisiae antibodies (ASCA) in coeliac disease. Gut 2006;55:296.

97. Mallant-Hent RCh MB, von Blomberg E, Yüksel Z, et al. Disappearance of anti-Saccharomyces cerevisiae antibodies in coeliac disease during a gluten-free diet. Eur J Gastroenterol Hepatol 2006;18:75-78.

98. Fresko I, Ugurlu S, Ozbakir F, et al. Anti-Saccharomyces cerevisiae antibodies (ASCA) in Behçet's syndrome. Clin Exp Rheumatol 2005;23:S67-70.

99. Toumi D, Mankaï A, Belhadj R, et al. Anti-Saccharomyces cerevisiae antibodies in coeliac disease. Scand J Gastroenterol 2007;42:821-6.

100. Monselise A, Weinberger A, Monselise Y, et al. Anti-Saccharomyces cerevisiae antibodies in Behçet's disease--a familial study. Clin Exp Rheumatol 2006;24:S87-90.

101. Condino AA, Hoffenberg EJ, Accurso F, et al. Frequency of ASCA seropositivity in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2005;41:23-6.

102. Hoffman IE, Demetter P, Peeters M, et al. Anti-saccharomyces cerevisiae IgA antibodies are raised in ankylosing spondylitis and undifferentiated spondyloarthropathy. Ann Rheum Dis 2003;62:455-9.

# APPENDIX I - PROFORMA

Case Number
Name

Age
Sex
Hospital Number
Address

Date of diagnosis

Final diagnosis and basis of diagnosis

Site of involvement

Harvey Bradshaw score for Crohn's Disease

ASCA IG G

## CLINICAL DETAILS

***Abdominal pain***

Duration
Location
Intensity
Character

***Diarrhoea***

Frequency
Volume

Blood/mucus

**Constitutional symptoms**
Anorexia
Weight loss
Fever
Others

**Anal canal lesions**
Fissures
Ulcers
Stenosis

***Extraintestinal manifestations***
Musculoskeletal Manifestations
Mucocutaneous Manifestations
Ocular Manifestations
Hepatobiliary Manifestations
Renal and Genitourinary Manifestations
Coagulation and Vascular Complications
Other Manifestations

*Complications ( intestinal/extraintestinal)*

*Surgeries*

*Past history*

*Personal history*

***Family histoy***

*Treatment history*

**Physical examination**

Height: Weight: BMI:
Pulse rate
Blood pressure
Pallor
Icterus
Clubbing
Lymphadenopathy
Oral hygiene
Skin
Perianal area

## *Systemic examination*

- Abdominal

- Musculoskeletal

- Respiratory

- Others

# *Investigations*

1. **CBC profile, ESR, CRP**

2. **Serum biochemistry**

3. **Radiologic findings**
   **CXR, U/S Abdomen, CT Abdomen**

4. **Endoscopy & Colonoscopy**

5. **Histologic examination**

6. **Others**